Volume 16 Number 5 1988 Nucleic Acids Research

The t(8;14) breakpoint of the EW 36 undifferentiated lymphoma cell line lies 5′ of MYC in a region prone to involvement in endemic Burkitt's lymphomas

Frank G.Haluska, Yoshihide Tsujimoto and Carlo M.Croce

The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104, USA

Received November 6, 1987; Revised and Accepted January 26, 1988

ABSTRACT

One of the best analyzed tumor-specific cytogenetic abnormalities is the t(8;14) chromosomal translocation observed in cases of Burkitt's and undifferentiated lymphomas (ULs), and acute lymphoblastic leukemias (ALLs). Here we analyze the cloned (8;14) chromosomal breakpoint of the UL cell line EW 36. We show that the region of chromosome 8 involved in the translocation is situated near a site previously demonstrated to harbor a cluster of endemic Burkitt's lymphoma breakpoints, approximatley 50 kb 5' of MYC. In those cases, we demonstrated that malfuction of the V-D-J recombinase generated the translocations. However, in this case the isotype switch mechanism of translocation is implicated: at the breakpoint, Sμ/Sγ and Cγ sequences are found on chromosome 14. Thus, the features of the EW 36 t(8;14) breakpoint are consonant with our model for B-cell lymphomagenesis which relates the precursor cell that gives rise to malignancy, the mechanism of translocation, and the phenotype of the tumor.

INTRODUCTION

Most malignancies, especially those of hematopoietic lineages, exhibit characteristic non-random chromosome abnormalities (1). At the molecular level of analysis, the most completely understood tumor-specific abnormalities are the chromosome translocations associated with Burkitt's lymphoma (2). Burkitt's lymphoma is a highly malignant B-cell neoplasm that occurs endemically in equatorial Africa and sporadically throughout the world (2). Over 80% of Burkitt's lymphomas exhibit t(8;14)(q24;q32) translocations which juxtapose the MYC proto-oncogene on chromosome 8 (3) and the immunoglobulin heavy chain (IgH) locus on chromosome 14 (4). Variant translocations account for the remaining 20% of Burkitt's lymphomas. These t(2;8) and t(8;22) translocations involve the MYC gene and the Ig light chain loci (2). All of these translocations, however, share a common feature. They place the MYC gene near genetic elements whose expression is normally strictly controlled in a B-cell specific fashion; consequently, MYC expression is dysregulated and proceeds constitutively (2,5). Other B-cell neoplasms, including undifferentiated lymphomas (ULs) and acute lymphoblastic leukemias (ALLs) display features quite similar to sporadic Burkitt's lymphomas (2).

Despite the apparent cytogenetic uniformity of the translocations observed in Burkitt's lymphoma, molecular analyses have revealed a large degree of heterogeneity. Chromosome breakpoints are scattered on all the involved chromosomes. Yet the distribution of breakpoints is non-random

and is roughly correspondent to the origin and phenotype of the tumor (6). Sporadic cases of Burkitt's lymphoma exhibit the following features: a relatively mature B-cell phenotype as evidenced by surface or secreted Ig (7,8), rearrangement of the MYC gene, and translocation involving switch regions on chromosome 14 (reviewed in 2). In contrast, endemic Burkitt's lymphoma cases are characterized by: a relatively immature B-cell phenotype (7,8), no detectable MYC rearrangement (9), and translocations involving J_H or D_H regions (10,11). These finding have led us to suggest that, in endemic cases of Burkitt's lymphoma, translocations arise as a consequence of V-D-J recombinase malfunction at an early stage of B-cell ontogeny; Epstein Barr Virus (EBV) infection facilitates this process by expanding the population of susceptible pre-B and immature B-cells (2,6,10). Sporadic cases probably occur at a much lower incidence due to rare switching mistakes in more mature B-cells (2).

We have previously described a region far 5' of MYC on chromosome 8 which is involved in the t(8;14) translocations of the endemic Burkitt's lymphoma cell lines P3HR-1 (10) and Daudi (11), as well as a pre-B-cell leukemia (10). Each of these translocations exhibits features implicating the V-D-J recombinase in its genesis. One might thus ask whether this mechanism of translocation is specific to the tumor type (i.e. pre-B cell) or specific to the involved region of chromosome 8 (i.e. far 5' of MYC). In the present study, we demonstrate that the t(8;14) breakpoint of the UL cell line EW 36 lies within 5 kilobases (kb) 5' of the P3HR-1 breakpoint on chromosome 8. We show that the translocation joined this region to the μ/γ switch on chromosome 14, which suggests that the mechanism of translocation is indeed cell type-specific. Finally, we discuss these findings in light of our hypothesis regarding oncogenesis in these and similar B-cell malignancies.

MATERIALS AND METHODS

Cell Lines

The cell line EW 36 was derived from an American case diagnosed as undifferentiated lymphoma and is well characterized (7,12). The EBV was not present in the tumor of origin, nor is it found in the cell line; both the tumor and cell line also carry the t(8;14) chromosome translocation (12). Characterization of the immunoglobulin expressed by the cell line indicated the presence of cell surface IgM and IgA (7), although a later study found no Ig secretion to occur (8).

Molecular Probes

The probes employed in this investigation derive from a region approximately 50 kb 5' of MYC on chromosome 8; they lie near a region prone to involvement in the t(8;14) translocations of endemic Burkitt's lymphomas (10,11,13). This region is illustrated in Fig. 1. We utilized p380j9 0.8 Ss, and 800 bp Sst I fragment in pUC 19 (10), and pD4A HH 1.6, a 1.6 kb Hind III fragment in pUC 19 (11), in the analysis of the EW 36 translocation. The Cγ probe has also been previously described (14).

Southern Blots

Genomic DNA was prepared by cell lysis with detergent and digestion with proteinase K as described (10). DNA was then digested with appropriate restriction endonucleases and separated electrophoretically through agarose gels. For genomic analyses, 10 µg was employed in each lane; for phage analyses, 750 ng was sufficient. Transfer to nitrocellulose filters was accomplished according to Southern (15). Blots were hybridized to nick-translated, ^{32}P-labeled plasmid probes as described (10).

Figure 1. Restriction map of region 5' of MYC. The relative orientation of the MYC gene and the chromosome 8 centromere are indicated. Probes are shown as black boxes above the map. The P3HR-1 endemic Burkitt's lymphoma and 380 Pre-B-cell leukemia breakpoints are located within pD4A HH 1.6 (10), and the Daudi endemic Burkitt's lymphoma breakpoint is situated about 1 kb 3' to the probe p380 6B B 2.7 (11). X, Xba I; Ss, Sst I; E, Eco RI; B, Bam HI; H, Hind III.

Molecular Cloning

An EW 36 genomic library was prepared in the λ phage EMBL 3 essentially as described (10,16). Briefly, high molecular weight DNA was partially digested with the restriction endonuclease Sau 3A, and fragments ranging from 15-23 kb in size were selected by fractionation over a 10-40% sucrose gradient. The appropriate fractions were ligated into EMBL 3. Approximately 6 x 10^5 phage plaques were screened with ^{32}P-labeled plasmid probe hybridizing phage purified and characterized by Southern analysis. Subcloning was accomplished by utilizing an electroelution apparatus (International Biotechnologies, Inc.) in the preparation of pure restriction fragments. Pure insert was then ligated into the plasmid pUC 19. Competent *E. coli* DH5a (BRL) was transformed with ligated DNA, white colonies were characterized, and plasmid DNA was prepared for further analysis (16).

DNA Sequencing

Nucleotide sequencing was accomplished using the dioxynucleotide chain termination method (17) on M13 phage clones of appropriate fragments of DNA (18).

RESULTS

Localization of the EW 36 t(8;14) Breakpoint by Southern Analysis

We took advantage of the availability of several probes in the region 5' of MYC to attempt to define the location of the EW 36 translocation breakpoint. A restriction map of a portion of the relevant region is illustrated in Fig. 1. We had previously demonstrated that the p380j9 0.8Ss probe detected rearrangements in two endemic Burkitt's lymphomas (10,11), and thus chose to test this probe against DNA from the EW 36 cell line as well. As shown in Fig. 2, Bam HI-digested EW 36 DNA demonstrates a rearrangement when probed with p380j9 0.8Ss. However, Hind III digestion reveals only a germline configuration (Fig. 2). Utilization of a second probe, pD4A HH 1.6, enabled us to more precisely identify the location of the breakpoint (Fig. 3). Digestion with Bam HI again reveals a rearrangement, although Sst I digestion does not (Fig. 3). These results place the breakpoint of EW 36 $14q^+$ chromosome 5' of pD4A on an approximately 20 kb Bam HI fragment.

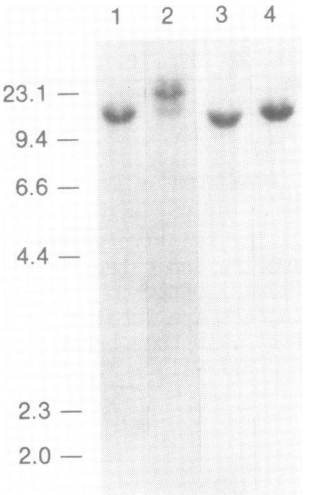

Figure 2. Southern blot of genomic DNAs probed with p380j9 0.8 Ss. Lane 1, placental DNA, Bam HI digest. Lane 2, EW 36 DNA, Bam HI digest. Lane 3, placental DNA, Hind III digest. Lane 4, EW 36 DNA, Hind III digest. Note the 20 kb rearranged band in lane 2. Molecular weight markers are indicated in kb.

Cloning of the Translocation Breakpoint

The finding that the breakpoint of EW 36, an EBV-negative undifferentiated lymphoma, was situated near the breakpoints of several cases of endemic Burkitt's lymphoma presented us with the opportunity to compare the molecular features of these translocations. To this end, we cloned the EW 36 t(8;14) breakpoint. Screening of approximately 600,000 recombinant phage clones yielded four positive plaques. These were analyzed by restriction enzyme digestion and Southern blotting. Two clones are illustrated schematically in Fig. 4. The clone which encompasses the site at which chromosomes 8 and 14 are joined (the $14q^+$ chromosome) is λE9B; λE7D represents the normal configuration of the corresponding chromosome 8 region. The two restriction maps are coincident to a point just to the right (5') of an Sst I site indicated by the arrow. This is the breakpoint. The map of the breakpoint clone is consistent with genomic Southern

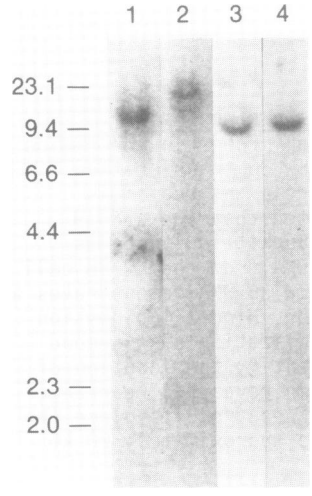

Figure 3. Southern blot probed with pD4A HH 1.6. Lane 1, placental DNA digested with Bam HI. Lane 2, EW 36 Bam HI digest. Lane 3, placental DNA, Sst I digest. Lane 4, EW 36, Sst I digest. These data place the EW 36 breakpoint farther 5' of MYC than pD4A HH 1.6.

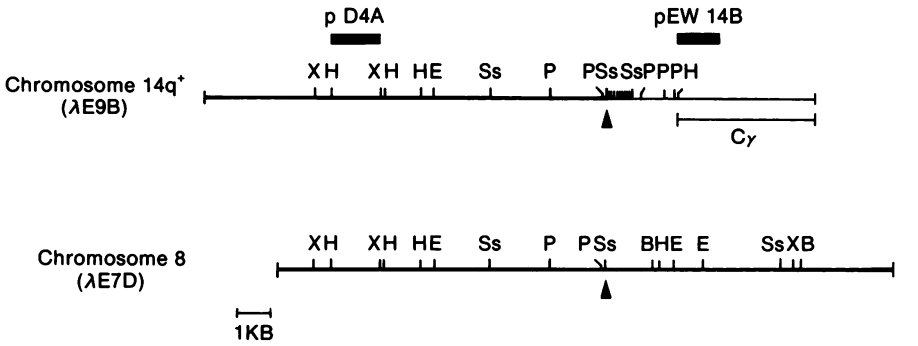

Figure 4. Restriction maps of recombinant phage clone insert DNAs encompassing the region surrounding the t(8;14) translocation. λ E9B corresponds to the $14q^+$ chromosome; λE7D, the normal chromosome 8. The arrowhead indicates the location of the breakpoint. To its left, these two clones share homology. To its right, λ E9B contains chromosome 14 sequences. The $14q^+$ breakpoint lies in the μ switch region which contains multiple Sst I sites; these are indicated by the vertical lines on λE9B. Homology to Cγ is also shown. Probes pD4A HH 1.6 and pEW 14B are indicated. P, Pst I. Not all Pst I sites are shown. Other restrictions site as for Fig. 1.

data, which indicated that the breakpoint was located on a large Bam HI fragment (Figs. 2 and 3).

To prove that we had indeed cloned the segment containing the junction between chromosomes 8 and 14, we subcloned a non-repetitive segment of DNA for use as a probe. This is designated pEW 14B in Fig. 4. Initially we probed a panel of DNAs from mouse/human somatic cell hybrid and found that

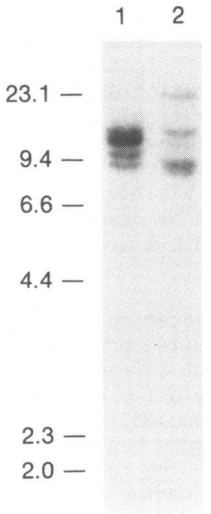

Figure 5. Southern blot of genomic DNA, probed with pEW 14B. Lane 1, placental DNA digested with Bam HI. The four Cγ regions are seen. In order of decreasing molecular weight, they are: Cγ2, 14.4 kb; Cγ1, 11.6 kb; Cγ3, 11.0 kb; Cγ4, 9.2 kb. Lane 2, EW 36 DNA digested with Bam HI. The bands can be identified as follows, also in order of decreasing molecular weight: The ~ 20 kb band corresponds to the translocated Cγ1 allele on the $14q^+$ chromosome. The 14.4 kb Cγ2 band is present, and its intensity suggests two germline copies. Next, a single germline 11.6 kb Cγ1 allele is seen. No germline Cγ3 allele is present. At about 9.2 kb, a germline Cγ4 band is apparent. Finally, at about 8 kb, a band is present which probably corresponds to a rearanged Cγ3 allele on the intact chromosome 14.

Figure 6. Nucleotide sequence of EW 36 t(8;14) breakpoint and the normal chromosome 8 region. Vertical lines indicate identity. The breakpoint is denoted by arrowheads. The sequences have been aligned to illustrate strong homology between the Sμ switch region motif, which is underlined, and the normal chromosome 8 sequence.

this probe mapped to chromosome 14 (results not shown). We then used pEW 14B to probe genomic Southern blots, as shown in Fig. 5. This probe recognizes segments of DNA corresponding to the γ constant regions (19), as was confirmed by probing the phage clones with a Cγ probe (results not shown). Fig. 5 also demonstrates that Bam HI-digested EW 36 DNA displays a rearranged band, when probed with pEW 14B, which corresponds to the rearranged band in Figs. 2 and 3. Thus, this single large fragment does contain both chromosome 8 and chromosome 14 sequences.

We next sought to determine the identity of the involved Cγ segment. The human genome contains four γ constant region genes as illustrated in Fig. 5 (19,20). The four constant regions are best resolved using Bam HI digestion; from largest to smallest, they can be ordered Cγ2, Cγ1, Cγ3, Cγ4 (19). In Fig. 5, lane 1, pEW 14B appears to correspond to Cγ1, based on the relative intensities of the four hybridization signals. This was confirmed by sequencing pEW 14B and comparing its sequence (not shown) to published Cγ sequences (19-23). With this information, we can reconstruct the configuration of the EW 36 γ genes. Fig. 5 (lane 2) demonstrates that one Cγ1 allele is rearranged (i.e., involved in the translocation on the $14q^+$ chromosome), and the other is in its germline configuration. Cγ1 and Cγ3 are linked (19), and the rearrangement in this case must have deleted one Cγ3 allele, since μ switch sequences are found at the breakpoint upstream of Cγ1 (see below). Since no Cγ3 germline band is visible, the other Cγ3 allele, which is on the normal chromosome 14, must be rearranged, yielding the approximately 8 kb band (Fig. 5). Both Cγ2 and Cγ4 are retained in their germline orientations.

Sequence of the Chromosome Breakpoint

To gain insight into the mechanism by which this translocation occurred, we obtained the nucleotide sequence across the t(8;14) breakpoint from clone λE9B. The breakpoint is situated on a 1.1 kb Pst I fragment of λE9B, and the corresponding normal region is encompassed by a 1.6 kb Pst I/Bam HI fragment of λE7D (Fig. 4). A portion of the nucleotide sequences of these fragments is shown in Fig. 6. The sequences begin with a common Sst I site, but diverge 39 nucleotides downstream. The remaining nucleotides on the $14q^+$ chromosome derive from the μ switch region (24).

Characteristic switch repeats are underlined. Note that substantial homology exists between the normal chromosome 8 sequence and the Sμ repeat units (Fig. 6). It thus appears likely that this translocation occurred as a consequence of aberrant operation of the Ig switch recombinase. The switch enzymes apparently joined the involved chromosome 8 sequences to the Sμ region during an attempted isotype switch. Subsequently, a switch involving Sμ and Sγ deleted the intervening chromosome 14 DNA and resulted in the configuration reflected by the breakpoint clones.

It is of interest that sequencing in this case was impeded by the presence of tandem repeats of an approximately 85 bp Sst I fragment on the $14q^+$ chromosome. Sequencing of several of these fragments (not shown) revealed differences of between three to five nucleotides between individual repeated segments. This repeat motif is apparently a unit of the μ switch. It is well known that the basic structure of the μ switch sequence includes G-G-G-N-G (G-A-G-C-T)n (ref. 24). The Sst I recognition site, G-A-G-C-T-C, is thus often included in the region.

DISCUSSION

B-cell neoplasias are a diverse group of disorders, and their clinical classification has historically been difficult. However, it is becoming clear that the most satisfactory conceptual classification for these malignancies considers primarily the underlying molecular pathology. In Burkitt's lymphoma and related tumors carrying t(8;14) translocations, two molecular presentations thus emerge (2,6). One type of tumor exhibits a rearranged MYC gene and joining of DNA segments near or within the MYC transcription unit to IgH switch sequences (2). By and large, these tumors occur with a sporadic distribution, are EBV-negative, and are comprised of B-cells which express and secrete large amounts of immunoglobulins (7,8,12). A second type of malignancy does not display a rearranged MYC gene (9). In these cases, joining between regions far 5' of MYC and upstream of IgH segments (D_H or J_H segments) is found (10,11). Generally, these tumors occur endemically in equatorial Africa, are EBV-positive, and secrete or express little immunogloublin (7,8,12). Thus, in most instances it is possible to relatively cleanly demarcate, on a molecular level, these two types of tumors.

This case is instructive because it presents an example of an exception which allows an examination of the importance of various factors in lymphomagenesis. We have demonstrated that EW 36, an undifferentiated EBV-negative tumor, in certain respects displays the molecular features of endemic Burkitt's lymphoma cases. We have shown that the chromosome 8 breakpoint is about 5 kb 5' of the P3HR-1 and Daudi breakpoints, far from the MYC gene. No structural alteration of the MYC transcriptional unit was observed. However, utilizing cloning and sequencing analysis we have also shown that the translocation occurred in a manner consistent with the aberrant operation of immunoglobulin isotype switching enzymes. This phenomenon has been demonstrated previously in several cases of Burkitt's lymphoma involving Sμ (25), Sγ (26) and Sα (27). It had always previously been described in conjunction with a rearranged MYC, and usually in sporadic cases of Burkitt's lymphoma (9). Thus study of this tumor revealed that despite its chromosome breakage in a region involved in endemic Burkitt's lymphoma translocations, the mechanism of translocation is consistent with its undifferentiated diagnosis and mature B-cell phenotype.

It is clear that the phenotype of EW 36 does not correlate with the region involved in translocation, nor with the fact that MYC is not rearranged. Rather, the biologic characteristics of the tumor correlate with the mechanism of translocation. We have previously proposed that the type of translocation mechanism utilized in a given tumor is an excellent indicator of the progenitor cell which gave rise to the malignancy (2,6,10). Thus, tumor phenotype derives from the differentiation state of the precursor cell involved in the malignant genetic change. Specifically, pre-B cells express functional V-D-J recombinase enzymes. In rare instances these recognize sequences on chromosome 8 5' of MYC. When this occurs, the t(8;14) translocation and endemic Burkitt's lymphoma ensues (10,11). Such mistakes may also give rise to other B-cell malignancies, including follicular lymphomas (28) and chronic lymphocytic leukemias (29). Sporadic or undifferentiated lymphomas, however, arise when more mature B-cells make switching mistakes. EW 36 falls into the latter category.

There are some discrepancies in the literature regarding the exact state of differentiation of the EW 36 cell line. The first characterization of EW 36 tumor and cell line cells demonstrated expression of IgM and IgA (7), while later analysis of the same cell line by the same group demonstrated no Ig expression (8). Thus, it is not clear exactly where the EW 36 cell line lies in the B-cell differentiation scheme. Moreover, switching enzyme activity can be demonstrated in pre-B-cell lines (32), suggesting that the window of B-cell maturation at which switching mistakes may occur is quite wide. However, EW 36 was established from a case diagnosed as an undifferentiated lymphoma. Its molecular features clearly support the correlation between the clinical presentation of tumors carrying t(8;14) translocations and the mechanism of chromosome translocation.

Our results also bear on the mechanism of MYC activation in t(8;14)-bearing tumors. Rearrangements of MYC in some sporadic tumors have engendered much speculation as to their primacy in the activation of MYC, despite the finding of tumors entirely lacking MYC alterations (30, and our unpublished results). That EW 36 carries a germline MYC gene, a normal MYC transcript by S1 analysis (31), and a translocation into a region far 5' of MYC, and yet appears phenotypically similar to other sporadic Burkitt's lymphomas, supports the proposition that MYC alterations occur secondarily in tumor evolution, and that *in cis* mechanisms are central to MYC activation (2,5).

ACKNOWLEDGEMENTS

The authors wish to thank Drs. M. Isobe and G. Russo for many insightful suggestions, and Ms. J. Romano and Mr. E. Emanuel for technical assistance. FGH is a Monica Shander Fellow at the Wistar Institute and is a trainee of the Medical Scientist Training Program at the University of Pennsylvania School of Medicine. This work was supported by grants from the National Institutes of Health, CA 39860 (CMC) and T32 CA 09171 (FGH).

REFERENCES

1. Yunis, J.J. (1983) Science 222, 227-236.
2. Haluska, F.G., Tsujimoto, Y. and Croce, C.M. (1987) Ann. Rev. Genet. 31, 321-345.
3. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. and Croce, C.M. (1982) Proc. Natl Acad. Sci. USA 79, 7824-7827.

4. Croce, C.M., Shander, M., Martinis, J., Cicurel, L., D'Ancona, G.G., Dolby, T.W. and Koprowski, H. (1979) Proc. Natl. Acad. Sci. USA 76, 3416-3419.
5. ar-Rushdi, A., Nishikura, K., Erikson, J., Watt, R., Rovera, G. and Croce, C.M. (1983) Science 222, 390-393.
6. Haluska, F.G., Tsujimoto, Y. and Croce, C.M. (1987) Trends Genet. 3, 11-15.
7. Magrath, I.T., Freeman, C.B., Pizzo, P., Gadek, J., Jaffe, E., Santaella, M., Hammer, C., Frank, M., Reaman, G. and Novikovs, L. (1980) J. Natl. Cancer Inst. 64, 477-483.
8. Benjamin, D., Magrath, I.T., Maguire, R., Janus, C., Todd, H.R. and Parsons, R.G. (1982) J. Immunol. 129, 1336-1342.
9. Pelicci, P.-G., Knowles, D.M. II, Magrath, I. and Dalla-Favera, R. (1986) Proc. Natl. Acad. Sci. USA 83, 2984-2988.
10. Haluska, F.G., Finver, S., Tsujimoto, Y. and Croce, C.M. (1986) Nature 324, 158-161.
11. Haluska, F.G., Tsujimoto, Y. and Croce, C.M. (1987) Proc. Natl. Acad. Sci. USA 84, 6835-6839.
12. Magrath, I.T., Pizzo, P.A., Whang-Pheng, J., Douglass, E.C., Alabaster, O., Gerber, P., Freeman, C.B. and Novikovs, L. (1980) J. Natl. Cancer Inst. 64, 465-476.
13. Haluska, F.G., Huebner, K. and Croce, C.M. (1987). Nucl. Acids Res. 15, 865.
14. Alexander, A., Steinmetz, M., Barritault, D., Fragone, B., Franklin, E., Hood, L. and Buxbaum, J. (1982) Proc. Natl. Acad. Sci. USA 79, 3260-3264.
15. Southern, E.M. (1979) J. Mol. Biol. 98, 503-517.
16. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
17. Sanger, F., Nicklen, S. and Coulson, A.E. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
18. Messing, J. (1983) Meth. Enzym. 101, 70-79.
19. Flanagan, J.G. and Rabbitts, T.H. (1982) Nature 300, 709-713.
20. Takahashi, N., Ueda, S., Obata, M., Nikaido, T., Nakai, S. and Honjo, T. (1982) Cell 29, 671-679.
21. Hamlyn, P.H. and Rabbitts, T.H. (1983) Nature 304, 135-139.
22. Krawinkel, U. and Rabbitts, T.H. (1982) EMBO J. 1, 403-407.
23. Ellison, J. and Hood, L. (1982) Proc. Natl. Acad. Sci. USA 79, 1984-1988.
24. Rabbitts, T.H., Forster, A. and Milstein, C.P. (1981) Nucl. Acids Res. 9, 4509-4524.
25. Gelmann, E.P., Psallidopoulos, M.C., Papas, T. and Dalla-Favera, R. (1983) Nature 306, 799-803.
26. Rabbitts, T.H., Hamlyn, P.H. and Baer, R. (1983) Nature 306, 760-765.
27. Showe, L.C., Ballantine, M., Nishikura, K., Erikson, J., Kaji, H. and Croce, C.M. (1985) Mol. Cell. Biol. 5, 501-509.
28. Tsujimoto, Y., Gorham, J., Cossman, J., Jaffe, E. and Croce, C.M. (1985) Science 229, 1390-1393.
29. Tsujimoto, Y., Jaffe, E., Cossman, J., Gorham, J., Nowell, P.C. and Croce, C.M. (1985) Nature 315, 340-343.
30. Showe, L.C., Moore, R.C.A., Erikson, J. and Croce, C.M. (1987) Proc. Natl. Acad. Sci. USA 84, 2824-2828.
31. Nishikura, K., Erikson, J., ar-Rushdi, A., Huebner, K. and Croce, C.M. (1985) Proc. Natl. Acad. Sci. USA 82, 2900-2904.
32. Ott, D.E., Alt, F.W. and Marcu, K.B. (1987). EMBO J 6, 577-584.